

Fungal Root Endophyte Colonization of Fern and Lycophyte Species from the Celaque National Park in Honduras

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ABSTRACT.—Root endophyte colonization was investigated in 32 fern and lycophyte species of 11 families collected from the Celaque National Park in Honduras. Arbuscular mycorrhizae (AM) were found in 11 plant species (34%) of Anemiaceae, Gleicheniaceae, Ophioglossaceae, Pteridaceae, Selaginellaceae, Thelypteridaceae, and Woodsiaceae. The abundance of arbuscular mycorrhizal fungi (AMF) in roots varied with particular species, ranging from 4% (*Sticherus underwoodianus*) to 78% (*Thelypteris patens*). The morphological AM colonization pattern of all investigated species was of the *Paris*-type. The mycelium of dark septate endophytes (DSE) was found in 19 species (58%), and was observed both in the roots of plants that were colonized by AMF and were devoid of AM association. However, in both cases the percentage of root colonization by these fungi was low. Exceptions were *Asplenium serra*, *Elaphoglossum erinaceum*, *Lellingeria prionodes*, and *Lycopodium thyoides*, where abundant DSE hyphae were observed. Our results are the first detailed report of both AMF and DSE associations of these plant species. Moreover, the mycorrhizal status of 27 plant species is reported for the first time.

KEY WORDS.—arbuscular mycorrhizae (AM), arbuscular mycorrhizal fungi (AMF), AM morphology, dark septate endophytes (DSE), ferns, *Paris*-type

Mycorrhizal symbiosis is the most widespread and commonly studied type of plant symbiotic associations. Nevertheless, the knowledge of mycorrhizae of ferns and lycophytes is scarce as only ca 10% of known species has been studied regarding their colonization by symbiotic fungi, namely arbuscular mycorrhizal fungi (AMF) (Glomeromycota) (Kessler *et al.*, 2009). There is a need to investigate mycorrhizae of ferns and lycophytes especially from tropical regions, where these groups of plants are most diverse and

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ecologically important, and where most major lineages of these plants have probably evolved (Kessler *et al.*, 2009, 2010). Moreover, little is known about associations of ferns and lycophytes with dark septate endophytes (DSE), a taxonomically and ecologically diverse group of fungi (Jumpponen, 2001). Among DSE, neutral, parasitic and symbiotic interactions with seed plants have been documented (Haselwandter and Read, 1982; Jumpponen, 2001; Wu and Guo, 2008); however, only few investigations have focused on their associations with ferns and lycophytes (Fernández *et al.*, 2008; Kessler *et al.*, 2009, 2010). In this study, we examined the fungal root endophyte colonization of fern and lycophyte species from the Celaque National Park in Honduras. We evaluated mycorrhizal status, the degree of AMF and DSE root colonization, and the morphotypes of arbuscular mycorrhiza (AM).

MATERIALS AND METHODS

Study area and material sampling.—The plant material for fungal root endophyte colonization assessment was collected from the Celaque National Park in Honduras in March/April 2008. The specimens were collected at various sites between 1000 m and 2800 m above sea level. We usually sampled one specimen per species except for four fern species, of which more than one sample was collected from the same habitat. In total, 34 plant taxa (32 species and two specimens assigned only to the genus level) from 11 families were sampled (Table 1). Whole plants were excavated and cleaned mechanically from the substrate. The roots were cut from the specimens, washed in water and stored in 50% ethanol. The plant specimens were deposited in the herbaria KRA (Institute of Botany, Jagiellonian University, Kraków, Poland) and TEFH (Universidad Nacional Autónoma de Honduras, Tegucigalpa, Honduras).

Root staining and the assessment of fungal colonization.—Roots were prepared according to the method of Phillips and Hayman (1970), with modifications. Roots were cleared in 10% KOH for 24 h and then rinsed in water. If the roots were still dark, the KOH was changed and the material was kept for further 24 hours, up to 72 h. The material was then acidified in 5% lactic acid in water (24 h), stained with 0.05% aniline blue in 80% lactic acid (72 h), and finally stored in 80% lactic acid until analysis. The whole procedure was performed at room temperature (22 °C). Root fragments (ca 1 cm) were mounted on slides in glycerol:lactic acid (5:1) and squashed using coverslides. The number of root fragments analyzed in the case of each specimen is reported in Table 1.

Fungal root colonization (AMF and DSE) was assessed using Nikon Eclipse 80i light microscope with Nomarski interference contrast optics and a digital camera with a panel for image analysis. The AMF colonization was identified on the basis of aseptate hyphae of irregular diameter, growing (i) intercellularly, forming arbuscules terminally in cortical cells (*Arum*-type of AM morphology), (ii) intracellularly with arbuscules developed on coils (*Paris*-type) or (iii) forming intermediate types (Dodd *et al.*, 2000; Smith and Read,

TABLE 1. Fungal root endophytes of fern and lycophyte species collected from the Celaque National Park.

Family ^a	Plant species ^b	Specimen root no. ^c	Habitat ^d	AM literature status ^e	AM parameters ^f				
					F	M	A	Fv	F _{DSE} ^g
Anemiaceae	<i>Anemia phyllitidis</i> (L.) Sw.	109 (30)	T	1+	100	77	76	24	12
	<i>Asplenium achilleifolium</i> (M. Martens & Galeotti) Liebm.	155 (30)	E	NS	-	-	-	-	7
Aspleniaceae	<i>Asplenium auriculatum</i> Sw.	153 (30)	E	NS	-	-	-	-	10
	<i>Asplenium fragrans</i> Sw.	117 (30)	E	NS	-	-	-	-	-
	<i>A. fragrans</i>	85 (30)	E	-	-	-	-	-	-
	<i>Asplenium praemorsum</i> Sw.	135 (30)	E	1,2-	-	-	-	-	-
	<i>Asplenium serra</i> Langsd. & Fisch.	156 (20)	E	NS	-	-	-	-	65
	<i>Asplenium</i> sp.	99 (30)	E	1+/-	-	-	-	-	43
	<i>Elaphoglossum erinaceum</i> (Fée) T. Moore	76 (20)	E	NS	-	-	-	-	100
Dryopteridaceae	<i>Elaphoglossum setigerum</i> (Sodirol) Diels	71 (10)	E	NS	-	-	-	-	-
Gleicheniaceae	<i>Diplazium bancroftii</i> (Hook.) A.R. Sm.	130 (15)	T	NS	47	12	4	20	100
	<i>Sticherus fulvus</i> (Desv.) Ching	113 (20)	T	NS	-	-	-	-	-
Lycopodiaceae	<i>Sticherus underwoodianus</i> (Maxon) Nakai	126 (17)	T	NS	29	4	3	-	12
	<i>Lycopodium thyoides</i> Humb. & Bonpl. ex Willd.	128 (10)	T	NS	-	-	-	-	100
Ophioglossaceae	<i>Botrychium virginicum</i> Willd.	147 (12)	T	1,3+	100	71	71	17	-
Polypodiaceae	<i>Campyloneurum amphotensonii</i> (Kunze ex Klotzsch) Fée	75 (20)	E	NS	-	-	-	-	-
	<i>Campyloneurum angustifolium</i> (Sw.) Fée	102 (25)	E	NS	-	-	-	-	8
Polypodiaceae	<i>C. angustifolium</i>	149 (30)	E	-	-	-	-	-	17
	<i>C. angustifolium</i>	157 (20)	E	-	-	-	-	-	-
	<i>Campyloneurum tenuipes</i> Maxon	116 (20)	E	NS	-	-	-	-	9
	<i>Lellingeria prionodes</i> (Mickel & Beitel) A.R. Sm. & R.C. Moran	83 (22)	E	NS	-	-	-	-	32
	<i>Niphidium crassifolium</i> (L.) Lellinger	96 (20)	T	NS	-	-	-	-	9
	<i>Pleopeltis mexicana</i> (Fée) Mickel & Beitel	74 (30)	E	NS	-	-	-	-	-
	<i>Pleopeltis polypodioides</i> (L.) E.G. Andrews & Windham	136 (30)	E	NS	-	-	-	-	-
Polypodiaceae	<i>Polypodium plebeium</i> Schltdl. & Cham.	138 (20)	E	NS	-	-	-	-	-
	<i>Polypodium pleurosorum</i> Kunze ex Mett.	150 (30)	E	NS	-	-	-	-	-
	<i>P. pleurosorum</i>	81 (30)	E	-	-	-	-	-	-
	<i>Polypodium subpetiolatum</i> Hook.	77 (10)	E	NS	-	-	-	-	-
	<i>Terpsichore</i> sp.	72 (18)	E	NS	-	-	-	-	-

TABLE 1. Continued.

Family ^a	Plant species ^b	Specimen root no. ^c	Habitat ^d	AM literature status ^e	AM parameters ^f			
					F	M	A	Fv F _{DSE} ^g
Pteridaceae	<i>Adiantum andicola</i> Liebm.	112 (10)	T	NS	80	53	53	10 20
	<i>A. andicola</i>	122 (10)	T		-	-	-	- 40
	<i>A. andicola</i>	78 (18)	T		100	73	67	- -
	<i>Cheliantes complanata</i> A.R. Sm.	73 (16)	T	NS	69	37	36	13 13
	<i>Pityrogramma ebenea</i> (L.) Proctor	114 (6)	T	NS	-	-	-	- 60
Selaginellaceae	<i>Selaginella pallescens</i> (C. Presl) Spring	101 (22)	T	NS	63	11	4	64 14
Thelypteridaceae	<i>Macrothelypteris torresiana</i> (Gaudich.) Ching	95 (20)	T	1+, 2+/-	90	65	65	85 -
	<i>Thelypteris patens</i> (Sw.) Small	121 (23)	T	NS	100	78	76	26 17
	<i>Thelypteris pilosula</i> (Klotzsch & H. Karst. ex Mett.) R.M. Tryon	98 (15)	T	NS	-	-	-	- -
	<i>Cystopteris fragilis</i> (L.) Bernh.	158 (16)	T	1,3+/-, 4+	100	68	61	50 38
	<i>Diplazium franconis</i> Liebm.	146 (22)	T	NS	55	11	10	- 9
Woodsiaceae								

^a Plant family names according to Smith *et al.*, 2006.
^b Plant species names mainly according to Nelson Sutherland *et al.*, 1996.
^c Specimen number (Naks *et al.*). Number of root fragments (ca 1 cm) analyzed is given in parenthesis after the specimen number.
^d Habitat: E – epiphytic, T – terrestrial.
^e Arbuscular mycorrhizal (AM) status of investigated fern and lycophyte species according to available literature: 1 – Wang and Qiu, 2006; 2 – Zhao, 2000; 3 – Berch and Kendrick, 1982; 4 – Cooper, 1976; (+) AM present, (-) AM absent, NS – not surveyed.
^f AM status observed in this study: mycorrhizal parameters [%]: F – mycorrhizal frequency, M – relative mycorrhizal root length, A – relative arbuscular richness; (-) AM fungi absent; Fv – frequency of the occurrence of vesicles, (-) vesicles absent.
^g F_{DSE} – frequency of the occurrence of dark septate endophyte (DSE) mycelium [%]; (-) DSE absent.

2008; Zubek *et al.*, 2008; Zubek and Błaszowski, 2009). The method proposed by Trouvelot *et al.* (1986) was followed for assessment of AM development. The parameters evaluated were: mycorrhizal frequency (F), relative mycorrhizal root length (M), and relative arbuscular richness (A). An estimate of mycorrhizal frequency (F%) is given as the ratio between root fragments colonized by AMF mycelium and the total number of root fragments analyzed. The relative mycorrhizal root length (M%) is an estimate of the amount of root cortex that is mycorrhizal relative to the whole root system. Arbuscule abundance (A%) is an estimate of arbuscule richness in the whole root system (Trouvelot *et al.*, 1986). DSE colonization was identified on the basis of regularly septate hyphae, usually dark pigmented, with facultatively occurring sclerotia (Jumpponen, 2001; Zubek *et al.*, 2008; Zubek and Błaszowski, 2009). The frequency of DSE mycelium occurrence in roots ($F_{DSE}\%$) was estimated as detailed above for AMF. Additionally, the frequency of the occurrence of resting spores of the fungi from the genus *Olpidium* ($F_{Olp}\%$) was assessed (Zubek *et al.*, 2008; Zubek and Błaszowski, 2009).

RESULTS

AM status.—Arbuscular mycorrhizae with arbuscules, which are the structural and functional criterion of the symbiosis, were found in 11 species (34%) from the families Anemiaceae, Gleicheniaceae, Ophioglossaceae, Pteridaceae, Selaginellaceae, Thelypteridaceae, and Woodsiaceae. The abundance of AMF in roots varied widely with particular taxa (Table 1). The highest AMF colonization was found in *Thelypteris patens* (M=78%) and *Anemia phyllitidis* (M=77%), which were also characterized by high arbuscule richness (A=76%). The lowest AMF colonization and arbuscule abundance were observed in *Sticherus underwoodianus* (M=4%, A=3%). In the case of *Adiantum andicola*, two of three analyzed root samples were mycorrhizal (M=53% and 73%), and one was devoid of AMF (Table 1).

All plant species showed *Paris*-type colonization in which AMF colonized roots by intracellular growth. Neighboring cortical cells contained hyphal coils on which arbuscules were formed (Fig. 1).

DSE colonization.—DSE were found in 19 identified species (58%) and in the roots of *Asplenium* sp. (Table 1). DSE colonization was observed in the roots of plants which were either colonized by AMF (9 specimens) or were devoid of AMF (13 specimens). Although the frequency of DSE occurrence in roots was above 30% in several species, the percentage of root colonization was low (data not shown). Single hyphae of different diameters, accompanied sporadically by sclerotia (Fig. 2), were found in the outer cortex and rhizodermis. Exceptions were *Asplenium serra*, *Elaphoglossum erinaceum*, *Lellingeria prionodes*, and *Lycopodium thyoides*, where DSE hyphae occurred abundantly in most analyzed root fragments (Fig. 2). DSE colonized up to 80% of root cortex of these plants. The mycelium was brownish or stained with aniline blue.

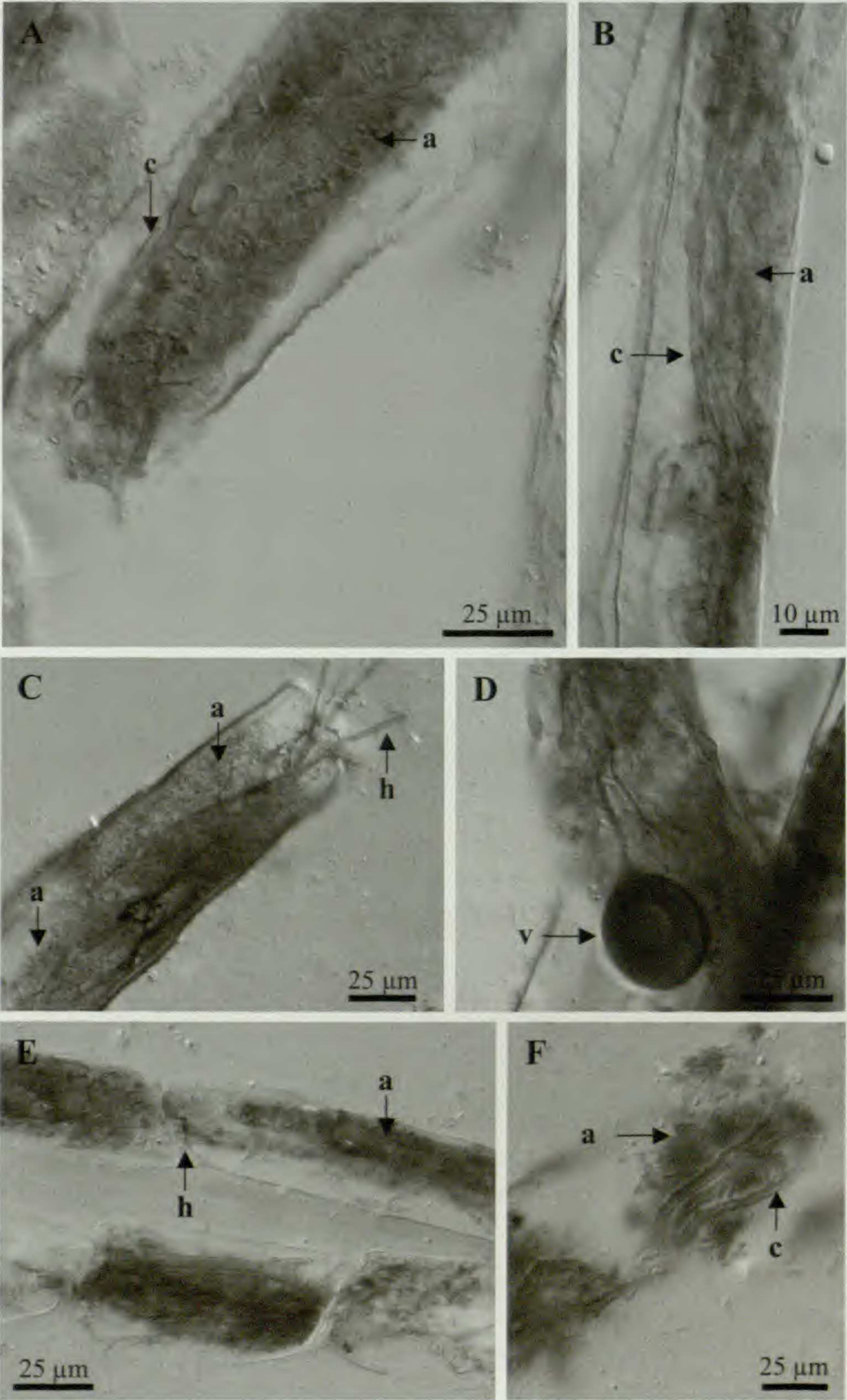


FIG. 1. A–F. Light micrographs of AMF mycelium in the root of *Cystopteris fragilis* (A, B), *Anemia phyllitidis* (C, D), and *Macrothelypteris torresiana* (E, F) (Paris-type); h – AMF hyphae growing intracellularly from cell to cell, a – arbuscules formed on coils, c – coils, v – vesicle. In the case of micrographs D and F, the AMF mycelium fell out the cells due to root squashing on slides.

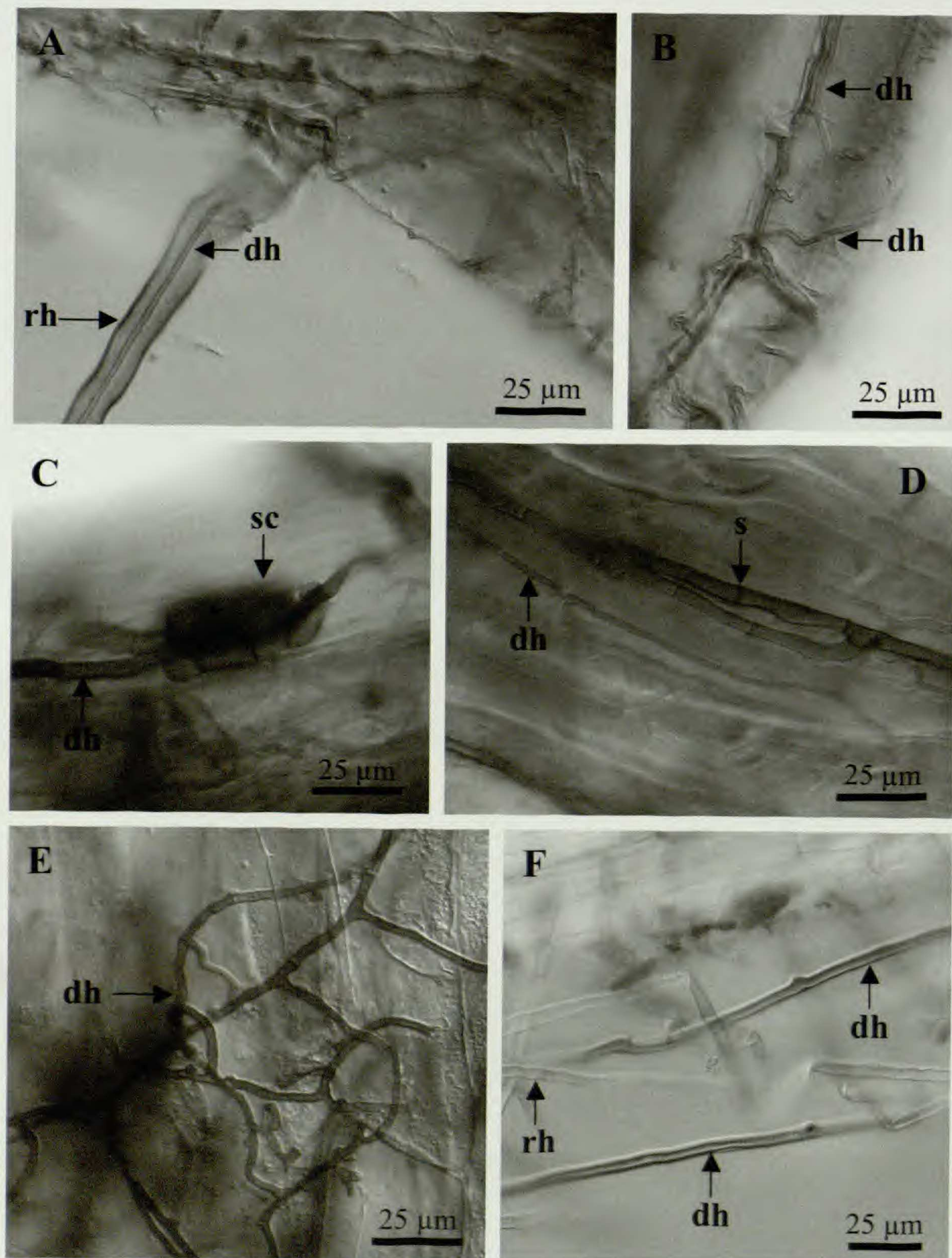


FIG. 2. A–F. Light micrographs of DSE mycelium observed in the root of *Elaphoglossum erinaceum* (A, B), *Adiantum andicola* (C), *Lycopodium thyoides* (D), and on the root surface of *Anemia phyllitidis* (E) and *Selaginella pallescens* (F); dh – DSE hyphae, sc – sclerotium, s – septa, rh – root hairs.

Single DSE hyphae were also detected on the root surface and/or between root hairs of 28 specimens (Fig. 2). In the old roots of several species, which were not included in the assessments of AMF and DSE colonization, DSE mycelium was abundant.

Other fungal endophytes.—From other fungal root endophytes, only *Polypodium pleurosorum* had resting spores of *Olpidium* spp., which were stained with aniline blue. Both the frequency of these structures ($F=20\%$) and the percentage of root infection were low.

DISCUSSION

In this paper, we present the detailed report of AMF and DSE associations in 32 fern and lycophyte species. The mycorrhizal status of 27 species is reported, to the best of our knowledge, for the first time. The presence of AMF in *Anemia phyllitidis* and *Botrychium virginicum*, as well as the absence of AMF in *Asplenium praemorsum* were confirmed, consistent with previous literature (Berch and Kendrick, 1982; Zhao, 2000; Wang and Qiu, 2006). *Cystopteris fragilis* and *Macrothelypteris torresiana* were highly colonized by AMF, being reported earlier as either mycorrhizal or devoid of AMF (Cooper, 1976; Berch and Kendrick, 1982; Zhao, 2000; Wang and Qiu, 2006). An obvious shortcoming of our study is the limitation of our investigations, in the case of most species, to one individual per species. Thus, our observations that particular species lack mycorrhizae do not mean that they are devoid of AMF at other sites and habitats. In order to clarify if a species lacks the symbiosis, or is facultatively or obligatory mycorrhizal, the collection of repetition samples from the same and different habitats is needed. Nevertheless, our investigations add data to the knowledge of mycorrhizal status and the biology and ecology of several fern and lycophyte species. Furthermore, as it was recognized for endangered seed plant species, studies on mycorrhizal associations may also be important in the context of ecological restoration, preservation, and propagation (Barroetavena *et al.*, 1998; Turnau and Haselwandter, 2002; Fuchs and Haselwandter, 2004; Zubek *et al.*, 2008, 2009).

Investigations on the occurrence of mycorrhizae in ferns and lycophytes indicate a considerable diversity of interactions, ranging from lack of symbiosis to facultative and obligate associations (Zhao, 2000; Turnau *et al.*, 2005; Fernández *et al.*, 2008; Kessler *et al.*, 2009, 2010). The positive response of ferns to AMF was confirmed in experimental research. In the studies carried out on *Pellaea viridis* (Forsk.) Prantl (Pellaeaceae), the presence of AMF in both gametophytes and sporophytes resulted in larger leaf area and root length of sporophytes in comparison to the plants devoid of AMF (Turnau *et al.*, 2005). As suggested earlier, the formation of mycorrhizae might be of great importance for epiphytic plants due to nutrient shortages under these conditions (Kessler *et al.*, 2009). However, we observed that all epiphytes were devoid of AMF. Although this survey, due to aforementioned limitations in specimen collection, does not allow definite conclusions about the relationship between AM presence and habitats, our findings are in

accordance with previous studies on ferns and angiosperms, where low AMF colonization or the absence of AMF were found among epiphytes (Lesica and Antibus, 1990; Maffia *et al.*, 1993; Michelsen, 1993; Kessler *et al.*, 2009, 2010). In this habitat, unsuitable growth conditions for AMF (the lack of well-developed soils) seem to be the reason for the absence or low AM colonization rather than a lack of functional need among epiphytes for mycorrhizal associations (Kessler *et al.*, 2009).

It is known that the AM morphology may depend on the host plant and fungal identity, and that different environmental factors may have an impact on AM pattern of root colonization (Cavagnaro *et al.*, 2001; Dickson, 2004; Yamato, 2004; Kubota *et al.*, 2005; Dickson *et al.*, 2007; Smith and Read, 2008). In the present study, all mycorrhizal plant species showed *Paris*-type of colonization. The results are in accordance with other studies where mostly this morphotype has been found among ferns and lycophytes (Zhang *et al.*, 2004; Dickson *et al.*, 2007). However, intermediate types, and less frequently *Arum* morphology of AM have also been documented (Dickson *et al.*, 2007; Fernández *et al.*, 2008; Kessler *et al.*, 2009). Our observations confirm earlier records that the Gleicheniaceae, Ophioglossaceae, Pteridaceae and Woodsiaceae (the genera *Cystopteris* and *Diplazium* included in the Athyriaceae family by Zhang *et al.*, 2004) only have the *Paris*-type. However, both intermediate and *Paris* patterns of AM colonization have been observed among species of the families Selaginellaceae and Thelypteridaceae (Zhang *et al.*, 2004; Dickson *et al.*, 2007). To the best of our knowledge, the AM morphology is reported for the first time for all investigated species and for the Anemiaceae family.

Dark septate endophytes (DSE) were found in the roots of several species, which is in accordance with earlier observations on ferns and lycophytes (Fernández *et al.*, 2008; Kessler *et al.*, 2009, 2010). DSE represent a taxonomically and ecologically diverse group of fungi. Among them, neutral, parasitic and symbiotic interactions with plants are known (Jumpponen, 2001). In the case of our study, it is difficult to judge the character of the DSE associations with the investigated species. Nevertheless, in the case of single DSE hyphae presence in a root system it seems unlikely that the fungus would have a significant impact on a plant. However, DSE hyphae occurred abundantly in the roots of *Asplenium serra*, *Elaphoglossum erinaceum*, *Lellingeria prionodes*, and *Lycopodium thyoides*, and an important influence of DSE on their hosts can not be excluded.

A positive response of angiosperms to DSE has been found in a few studies. DSE isolates were shown to stimulate plant growth or increase phosphorus concentration in two *Carex* species (Haselwandter and Read, 1982). DSE were also found to colonize roots of *Saussurea involucrata* Kar. et Kir. ex Maxim (Asteraceae). In a laboratory experiment, seedlings of this species were inoculated with a DSE strain previously isolated from *S. involucrata* roots; the plants were colonized heavily and displayed enhanced growth due to the presence of DSE fungus (Wu and Guo, 2008). In order to reveal the nature of DSE associations with ferns and lycophytes, further research is necessary under experimental conditions. To our knowledge, such studies have not been conducted so far.

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LITERATURE CITED

- BARROETAVENA, C., S. D. GISLER, D. L. LUOMA and R. J. MEINKE. 1998. Mycorrhizal status of the endangered species *Astragalus applegatei* Peck as determined from a soil bioassay. *Mycorrhiza* 8:117–119.
- BERCH, S. M. and B. KENDRICK. 1982. Vesicular-arbuscular mycorrhizae of southern Ontario ferns and fern-allies. *Mycologia* 74:769–776.
- CAVAGNARO, T. R., L.-L. GAO, F. A. SMITH and S. E. SMITH. 2001. Morphology of arbuscular mycorrhizas is influenced by fungal identity. *New Phytol.* 151:469–475.
- COOPER, K. M. 1976. A field survey of mycorrhizas in New Zealand ferns. *New Zeal. J. Bot.* 14:169–181.
- DICKSON, S. 2004. The *Arum-Paris* continuum of mycorrhizal symbioses. *New Phytol.* 163:187–200.
- DICKSON, S., A. SMITH and S. E. SMITH. 2007. Structural differences in arbuscular mycorrhizal symbioses: more than 100 years after Gallaud, where next? *Mycorrhiza* 17:375–393.
- DODD, J. C., C. L. BODDINGTON, A. RODRIGUEZ, C. GONZÁLEZ-CHÁVEZ and I. MANSUR. 2000. Mycelium of arbuscular mycorrhizal fungi (AMF) from different genera: form, function and detection. *Plant Soil* 226:131–151.
- FERNÁNDEZ, N., M. I. MESSUTI and S. FONTENLA. 2008. Arbuscular mycorrhizas and dark septate fungi in *Lycopodium paniculatum* (Lycopodiaceae) and *Equisetum bogotense* (Equisetaceae) in a Valdivian temperate forest of Patagonia, Argentina. *Am. Fern J.* 98:117–127.
- FUCHS, B. and K. HASELWANDTER. 2004. Red list plants: colonization by arbuscular mycorrhizal fungi and dark septate endophytes. *Mycorrhiza* 14:277–281.
- HASELWANDTER, K. and D. J. READ. 1982. The significance of a root-fungus association in two *Carex* species of high-alpine plant communities. *Oecologia* 53:352–354.
- JUMPPONEN, A. 2001. Dark septate endophytes – are they mycorrhizal? *Mycorrhiza* 11:207–211.
- KESSLER, M., R. JONAS, D. CICUZZA, J. KLUGE, K. PIĄTEK, P. NAKS and M. LEHNERT. 2009. A survey of the mycorrhization of Southeast Asian ferns and lycophytes. *Plant Biol.* doi:10.1111/j.1438-8677.2009.00270.x.
- KESSLER, M., R. JONAS, D. STRASBERG and M. LEHNERT. 2010. Mycorrhizal colonization of ferns and lycophytes on the island of La Réunion in relation to nutrient availability. *Basic Appl. Ecol.* (in press).
- KUBOTA, M., T. P. MCGONIGLE and M. HYAKUMACHI. 2005. Co-occurrence of *Arum*- and *Paris*-type morphologies of arbuscular mycorrhizae in cucumber and tomato. *Mycorrhiza* 15:73–77.
- LESICA, P. and R. K. ANTIBUS. 1990. The occurrence of mycorrhizae in vascular epiphytes of two Costa Rican rain forests. *Biotropica* 33:250–258.
- MAFFIA, B., N. M. NADKARNI and D. P. JANOS. 1993. Vesicular-arbuscular mycorrhizae of epiphytic and terrestrial Piperaceae under field and greenhouse conditions. *Mycorrhiza* 4:5–9.
- MICHELSSEN, A. 1993. The mycorrhizal status of vascular epiphytes in Bale Mountains National Park, Ethiopia. *Mycorrhiza* 4:11–15.
- NELSON SUTHERLAND, C., R. GAMARRA GAMARRA and J. FERNANDEZ CASAS. 1996. Hondurensis Plantarum Vascularum Catalogus. Pteridophyta. *Fontqueria* 43:1–143.
- PHILLIPS, J. and D. S. HAYMAN. 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *T. Brit. Mycol. Soc.* 55:158–161.
- SMITH, A. R., K. M. PRYER, E. SCHUETTPELZ, P. KORALL, H. SCHNEIDER and P. G. WOLF. 2006. A classification for extant ferns. *Taxon* 55:705–731.
- SMITH, S. E. and D. J. READ. 2008. *Mycorrhizal symbiosis. Third edn.* Academic Press, London.

- TROUVELOT, A., J. L. KOUGH and V. GIANINAZZI-PEARSON. 1986. Mesure du taux de mycorhization VA d'un système racinaire. Recherche de méthodes d'estimation ayant une signification fonctionnelle. Pp. 217–221, *In*: V. Gianinazzi-Pearson and S. Gianinazzi, eds. *Physiological and genetical aspects of mycorrhizae*. INRA, Paris.
- TURNAU, K. and K. HASELWANDTER. 2002. Arbuscular mycorrhizal fungi, an essential component of soil microflora in ecosystem restoration. Pp. 137–149, *In*: S. Gianinazzi, H. Schüepp, J. M. Barea and K. Haselwandter, eds. *Mycorrhizal technology in agriculture. From genes to mycorrhiza application*. Birkhauser Verlag, Switzerland.
- TURNAU, K., T. ANIELSKA and A. JURKIEWICZ. 2005. Mycothallic/mycorrhizal symbiosis of chlorophyllous gametophytes and sporophytes of a fern, *Pellaea viridis* (Forsk.) Prantl (Pellaeaceae, Pteridales). *Mycorrhiza* 15:121–128.
- WANG, B. and Y. L. QIU. 2006. Phylogenetic distribution and evolution of mycorrhizas in land plants. *Mycorrhiza* 16:299–363.
- WU, L. and S. GUO. 2008. Interaction between an isolate of dark-septate fungi and its host plant *Saussurea involucrata*. *Mycorrhiza* 18:79–85.
- YAMATO, M. 2004. Morphological types of arbuscular mycorrhizal fungi in roots of weeds on vacant land. *Mycorrhiza* 14:127–131.
- ZHANG, Y., L. D. GUO and R. J. LIU. 2004. Arbuscular mycorrhizal fungi associated with common pteridophytes in Dujiangyan, southwest China. *Mycorrhiza* 14:25–30.
- ZHAO, Z-W. 2000. The arbuscular mycorrhizas of pteridophytes in Yunnan, southwest China: evolutionary interpretations. *Mycorrhiza* 10:145–149.
- ZUBEK, S., K. TURNAU and J. BŁASZKOWSKI. 2008. Arbuscular mycorrhiza of endemic and endangered plants from the Tatra Mts. *Acta Soc. Bot. Pol.* 77:149–156.
- ZUBEK, S. and J. BŁASZKOWSKI. 2009. Medicinal plants as hosts of arbuscular mycorrhizal fungi and dark septate endophytes. *Phytochem. Rev.* 8:571–580.
- ZUBEK, S., K. TURNAU, M. TSIMILLI-MICHAEL and R. J. STRASSER. 2009. Response of endangered plant species to inoculation with arbuscular mycorrhizal fungi and soil bacteria. *Mycorrhiza* 19:113–123.